PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 22 October 1999 (22.10.99)	
International application No. PCT/GB99/00742	**Applicant's or agent's file reference** P/4317.WO
International filing date (day/month/year) 12 March 1999 (12.03.99)	**Priority date** (day/month/year) 12 March 1998 (12.03.98)
Applicant HASSARD, John, Dr.	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

09 September 1999 (09.09.99)

[] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Lazar Joseph Panakal
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

eiving office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* P/4317.WO

Box No. I TITLE OF INVENTION

ANALYSER

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE
Sherfield Building
Exhibition Road
London, SW7 2AZ
United Kingdom

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(i.e. country)* of nationality: United Kingdom

State *(i.e. country)* of residence: United Kingdom

This person is applicant for the purposes of: ☐ all designated States ☑ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (i.e. country) of residence if no State of residence is indicated below.)*

HASSARD, Dr John
Flat 3
Selkirk Hall
Princes Garden
London, SW7 1LU

This person is:

☐ applicant only

☑ applicant and inventor

☐ inventor only *(if this check-box is marked, do not fill in below)*

State *(i.e. country)* of nationality: United Kingdom

State *(i.e. country)* of residence: United Kingdom

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☑ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicant and/or (further) inventors are indicated on a continuation sheet

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☑ agent ☐ common representative

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)*

TURNER, James Arthur ET AL
21 New Fetter Lane
London, EC4A 1DA
United Kingdom

Telephone No. +44 1703 634816

Facsimile No. +44 1703 224262

Teleprinter No. 477667 YOUNGS G

☐ Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked)*:

Regional Patent

- ☑ AP **ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☑ EA **Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikstan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☑ EP **European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☑ OA **OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Máli, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if any other kind of protection or treatment desired, please specify on dotted line)*

National Patent *(if other kind of protection or treatment desired, specify on dotted line)*:

- ☑ AL Albania
- ☑ AM Armenia
- ☑ AT Austria
- ☑ AU Australia
- ☑ AZ Azerbaijan
- ☑ BA Bosnia and Herzegovina
- ☑ BB Barbados
- ☑ BG Bulgaria
- ☑ BR Brazil
- ☑ BY Belarus
- ☑ CA Canada
- ☑ CH AND LI Switzerland and Liechtenstein
- ☑ CN China
- ☑ CU Cuba
- ☑ CZ Czech Republic
- ☑ DE Germany
- ☑ DK Denmark
- ☑ EE Estonia
- ☑ ES Spain
- ☑ FI Finland
- ☑ GB United Kingdom
- ☑ GE Georgia
- ☑ GH Ghana
- ☑ GM Gambia
- ☑ GW Guinea-Bissau
- ☑ HU Hungary
- ☑ ID Indonesia
- ☑ IL Israel
- ☑ IS Iceland
- ☑ JP Japan
- ☑ KE Kenya
- ☑ KG Krygyzstan
- ☑ KP Democratic People's Republic of Korea
- ☑ KR Republic of Korea
- ☑ KZ Kazakstan
- ☑ LC Saint Lucia
- ☑ LK Sri Lanka
- ☑ LR Liberia
- ☑ LS Lesotho
- ☑ LT Lithuania
- ☑ LU Luxembourg
- ☑ LV Latvia
- ☑ MD Republic of Moldova
- ☑ MG Madagascar
- ☑ MK The former Yugoslav Republic of Macedonia
- ☑ MN Mongolia
- ☑ MW Malawi
- ☑ MX Mexico
- ☑ NO Norway
- ☑ NZ New Zealand
- ☑ PL Poland
- ☑ PT Portugal
- ☑ RO Romania
- ☑ RU Russian Federation
- ☑ SD Sudan
- ☑ SE Sweden
- ☑ SG Singapore
- ☑ SI Slovenia
- ☑ SK Slovakia
- ☑ SL Sierra Leone
- ☑ TJ Tajikstan
- ☑ TM Turkmenistan
- ☑ TR Turkey
- ☑ TT Trinidad and Tobago
- ☑ UA Ukraine
- ☑ UG Uganda
- ☑ US United States of America
- ☑ UZ Uzbekistan
- ☑ VN Viet Nam
- ☑ YU Yugoslavia
- ☑ ZW Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after the issuance of this sheet:

- ☑ HR Croatia
- ☑ GD Grenada
- ☑ IN India
- ✓ AE United Arab Emirates

In addition to the designations made above, the applicant also makes under Rule 4.9(b) all designations which would be permitted under the PCT except the designation(s) of

The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit.
(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)

Box No. VI PRIORITY CLAIM

Further priority claims are indicated in the Supplemental Box ☐

The priority of the following earlier application(s) is hereby claimed:

Country *(in which, or for which, the application was filed)*	Filing Date *(day/month/year)*	Application No.	Office of filing *(only for regional or international application)*
item (1) UK	12 Mar 1998	9805301.0	
item (2)			
item (3)			

Mark the following check-box if the certified copy of the earlier application is to be issued by the Office which for the purposes of the present international application is the receiving Office (a fee may be required):

☑ The receiving Office is hereby requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) identified above as item(s) : 1

Box No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):* ISA / _____

Earlier Search *Fill in where a search (international, international-type or other) by the International Search Authority has already been carried out or requested and the Authority is now requested to base the international search, to the extent possible, on the results of that earlier search. Identify such search or request either by reference to the relevant application (or the translation thereof) or by reference to the search request:*

Country (or regional Office): Date *(day/month/year)* Number:

Box No. VII CHECK LIST

This international application contains the following number of sheets:

1. request	: 3	sheets
2. description	: 6	sheets
3. claims	: 2	sheets
4. abstract	: 1	sheets
5. drawings	: 4	sheets
Total	: 16	sheets

This international application is accompanied by the item(s) marked below:

1. ☐ separate signed power of attorney
2. ☑ copy of general power of attorney 1
3. ☐ statement explaining lack of signature
4. ☐ priority documents(s) *identified in Box No. VI as item(s):*
5. ☑ fee calculation sheet
6. ☐ separate indications concerning deposited microorganisms
7. ☐ nucleotide and/or amino acid sequence listing (diskette)
8. ☐ other *(specify):*

Figure No. 1 of the drawings (if any) should accompany the abstract when it is published

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request)

For receiving Office use only

1. Date of actual receipt of the purported international application:
2. Drawings: ☐ received: ☐ not received:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:
4. Date of timely receipt of the required corrections under PCT Article 11(2):
5. International Searching Authority specified by the applicant: ISA /
6. ☐ Transmittal of search copy delayed until search fee paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

16

ATENT COOPERATION TREATY

REC'D 19 JUN 2000
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P/4317.WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/00742	12/03/1999	12/03/1998
International Patent Classification (IPC) or national classification and IPC G01N27/447		
Applicant IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

 ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

 These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

 - I ☒ Basis of the report
 - II ☐ Priority
 - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV ☐ Lack of unity of invention
 - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
 - VI ☐ Certain documents cited
 - VII ☒ Certain defects in the international application
 - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09/09/1999	15.06.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Müller, T Telephone No. +49 89 2399 2285

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/00742

I. Basis of th report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-6 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims 3
	No:	Claims 1,2
Inventive step (IS)	Yes:	Claims 3
	No:	Claims 4-17
Industrial applicability (IA)	Yes:	Claims 1-17
	No:	Claims

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

D1: EP-A-0 708 331 (HEWLETT PACKARD CO) 24 April 1996
D2: WO 98 10122 A (UNIV NORTHEASTERN) 12 March 1998
D3: MACDONALD J H ET AL: 'A CCD system for UV imaging of biomolecules' NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH, SECTION - A: ACCELERATORS, SPECTROMETERS, DETECTORS AND ASSOCIATED EQUIPMENT, vol. 392, no. 1-3, 21 June 1997, page 227-232 XP004090959

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Technical field:
 The application is related to a micro fabricated capillary electrophoresis device for DNA analysis in an automated or semi-automated manner.

2. Prior art:
 Closest prior art document D1 discloses a miniaturized total analysis system (μ-TAS) fully contained on a single planar surface (page 4, line 35-36). The device according to D1 comprises a substrate of polymer material, an array of elongate capillary channels formed in the substrate, (page 10, line 33-50), means for driving a sample to be tested along the channels (page 11, line 39-42), a radiation source and a radiation detector disposed on either side of the channel array and the substrate may be formed from materials such as quartz sapphire, diamond and fused silica (page 7, line 16-48).

3. Novelty:
 As a consequence the subject-matter of claims 1 and 2 is not new over the prior art according to D1 (Article 33 (2) PCT).

 An analyser with an array of capillary channels according to claim 1 in which the substrate is formed of sapphire having a coating of nanocrystalline diamond is not disclosed in any of the prior art documents cited in the search report. Therefore the subject-matter of claim 3 is new as required by Article 33 (2) PCT.

4. Inventive step:
Insofar dependent claims 4-17 refer to claim 1 or 2, the solution proposed in claims 4-17 of the present application cannot be considered as involving an inventive step (Article 33(3) PCT) for the following reasons.

In claims 4-17 details of a micro analyser device according to D1 are defined which come within the scope of the customary practice followed by persons skilled in the art, especially as the advantages thus achieved can readily be foreseen. Consequently, the subject-matter of claim 4-17 also lacks an inventive step.

The solution according to claim 3 is not hinted by the available prior art. As a consequence the subject-matter of claim 3 involves an inventive step as required by Article 33 (3) PCT.

Insofar dependent claims 4-17 refer back to the subject-matter of claim 3, dependent claims 4-17 meet also the requirements of the PCT.

Re Item VII

Certain defects in the international application

1. Independent claim 1 is not in the two-part form in accordance with Rule 6.3(b) PCT, which in the present case would be appropriate, with those features known in combination from the prior art (document D1) being placed in the preamble (Rule 6.3(b)(i) PCT) and with the remaining features being included in the characterising part (Rule 6.3(b)(ii) PCT).

2. The features of the claims are not provided with reference signs placed in parentheses (Rule 6.2(b) PCT).

Re Item VIII

Certain observations on the international application

1. The subject-matter of claim 1 is not clear because an array of channels cannot be formed by one elongate capillary channel (Article 6 PCT).

ATENT COOPERATION TREAT

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
P/4317.WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 00742	12/03/1999	12/03/1998
Applicant		
IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[] contained in the international application in written form.

[] filed together with the international application in computer readable form.

[] furnished subsequently to this Authority in written form.

[] furnished subsequently to this Authority in computer readble form.

[] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [] **Certain claims were found unsearchable** (See Box I).

3. [] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

CAPILLARY ELECTROPHORESIS DEVICE

5. With regard to the **abstract,**

[] the text is approved as submitted by the applicant.

[X] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. 1

[X] as suggested by the applicant.

[] None of the figures.

[] because the applicant failed to suggest a figure.

[] because this figure better characterizes the invention.

Box III TEXT OF THE ABSTRACT (Continuation of item 5 of the first sheet)

An analyser comprises a substrate (20) of diamond, sapphire or a polymer material; an array (10) of elongate capillary channels formed in the substrate; means (40) for driving a sample to be tested along one or more of the channels whereby the velocities of components of the sample along the channels depends on the relative molecular weights of those components; and a radiation source (100) and a radiation detector (30) disposed on either side of the channel array so as to detect the presence of material in the channels as interruptions in the radiation path between the radiation source and the radiation detector.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 99/00742

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 708 331 A (HEWLETT PACKARD CO) 24 April 1996 see page 4, line 21 - page 5, line 14 see page 6, line 1 - page 10, line 26 ---	1,2
X,P	WO 98 10122 A (UNIV NORTHEASTERN) 12 March 1998 see the whole document --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 1999	30/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, T

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MACDONALD J H ET AL: "A CCD system for UV imaging of biomolecules" NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH, SECTION - A: ACCELERATORS, SPECTROMETERS, DETECTORS AND ASSOCIATED EQUIPMENT, vol. 392, no. 1-3, 21 June 1997, page 227-232 XP004090959 see page 227, left-hand column - page 229, right-hand column; figure 2 -----	1,8-11, 14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
/GB 99/00742

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0708331 A	24-04-1996	US 5500071 A	19-03-1996
		US 5571410 A	05-11-1996
		EP 0708330 A	24-04-1996
		EP 0734281 A	02-10-1996
		EP 0734282 A	02-10-1996
		JP 9508706 T	02-09-1997
		WO 9612545 A	02-05-1996
		WO 9612546 A	02-05-1996
		US 5658413 A	19-08-1996
		US 5641400 A	24-06-1997
		US 5882571 A	16-03-1999
		US 5804022 A	08-09-1998
		JP 8334505 A	17-12-1996
WO 9810122 A	12-03-1998	NONE	

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 27/447 — A1

(11) International Publication Number: WO 99/46590

(43) International Publication Date: 16 September 1999 (16.09.99)

(21) International Application Number: PCT/GB99/00742

(22) International Filing Date: 12 March 1999 (12.03.99)

(30) Priority Data:
9805301.0 12 March 1998 (12.03.98) GB

(71) Applicant *(for all designated States except US)*: IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY & MEDICINE [GB/GB]; Sherfield Building, Exhibition Road, London SW7 2AZ (GB).

(72) Inventor; and

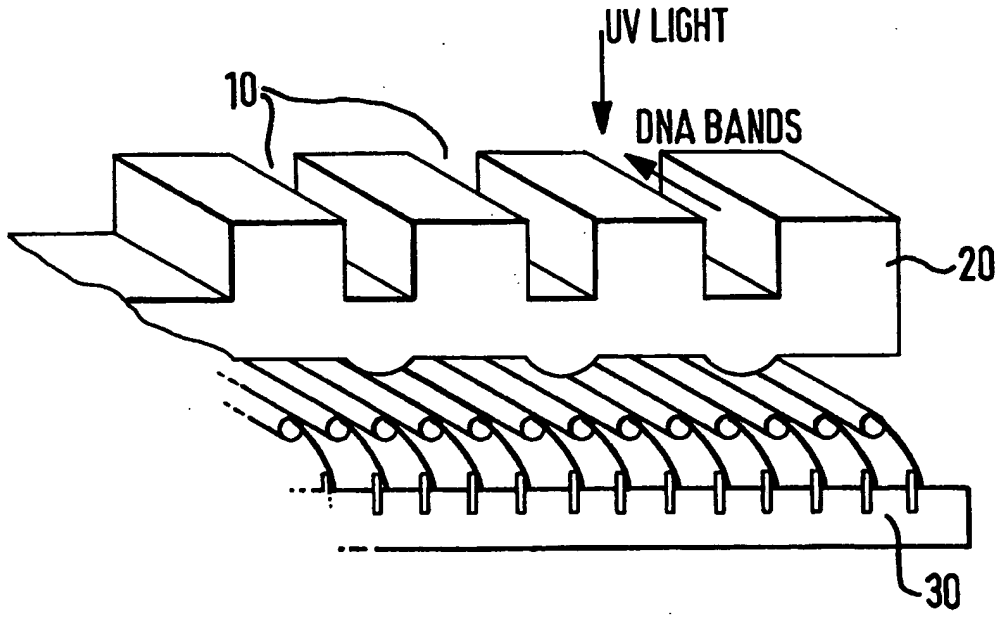
(75) Inventor/Applicant *(for US only)*: HASSARD, John, Dr. [GB/GB]; Flat 3, Selkirk Hall, Princes Garden, London SW7 1LU (GB).

(74) Agent: TURNER, James, Arthur; 21 New Fetter Lane, London EC4A 1DA (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: CAPILLARY ELECTROPHORESIS DEVICE



(57) Abstract

An analyser comprises a substrate (20) of diamond, sapphire or a polymer material; an array (10) of elongate capillary channels formed in the substrate; means (40) for driving a sample to be tested along one or more of the channels whereby the velocities of components of the sample along the channels depends on the relative molecular weights of those components; and a radiation source (100) and a radiation detector (30) disposed on either side of the channel array so as to detect the presence of material in the channels as interruptions in the radiation path between the radiation source and the radiation detector.

CAPILLARY ELECTROPHORESIS DEVICE

This invention relates to analysers. Embodiments of the invention relate to instruments for the determination of molecular characteristics, such as the length of nucleic acid in terms of base pairs, and the sequencing of genetic samples.

There are several current techniques for analysing materials such as DNA samples in an automated or semi-automated manner. One such technique which has been demonstrated for DNA analysis is the use of so-called microfabricated capillary electrophoresis (CE) chips.

These devices comprise a substrate in which a number of very fine capillary channels are etched and filled with a gel material. A material to be analysed passes along the capillary channel under the influence of an electric field. Components of the material - for example, nucleic acids - progress along the channel at different rates depending on the relative molecular weights of the components, leading to a separation by molecular weight.

Current techniques use photographic techniques to image radioactive or luminescent tags attached to the nucleic acids. This is a time consuming process. A quicker but expensive alterative is to use phosphor imagers to record the sequences. Furthermore, both processes use hazardous chemicals to tag the nucleic acids and the safe use and disposal of these is a major problem, requiring skilful scientific and technical input.

As mentioned above, all of these techniques are relatively slow. In any current emission technique, such as CE-laser induced fluorescence (CE-LIF) the time-to-sequence depends on the separation gradient (i.e. the electric field) and the discriminator power (e.g. the capillary or electrophoresis gel) convolved with the size of the objects to be separated.

There is a need for an improved technique offering a faster response than current techniques and avoiding the use of hazardous materials.

PCT/GB96/01121 discloses an electrophoresis system in which material components are driven along quartz tubes. In effect, the shadow of the separated components is detected by directing an ultraviolet (UV) light from one side of the tubes towards a detector at the other side.

This invention provides an analyser comprising: a substrate of diamond, sapphire or a polymer material; an array of one or more elongate capillary channels formed in the substrate; means for driving a sample to be tested along one or more of the channels whereby the velocities of components of the sample along the channels depends on the relative molecular weights of those components; a radiation source and a radiation detector disposed on either side of the channel array so as to detect the presence of material in the channels as interruptions in the radiation path between the radiation source and the radiation detector.

The invention addresses the above problems by providing a new selection of substrates offering many advantages over the glass, quartz and plastics of previous analysers. In particular, the whole apparatus can be miniaturised and the use of hazardous markers is avoided by detecting the "shadow" of separated components (e.g. DNA fragments).

Embodiments of the invention can provide an analysis technique which can be carried out while avoiding the use of mutagenic, toxic and carcinogenic chemiluminescent, bioluminescent and radiolabels, with associated benefits in running costs, safety and ease of disposal.

Embodiments of the invention can provide up to an order of magnitude increase in speed for DNA sequencing. For example, a sequence of 500 base pairs which might take 6 hours using a conventional electrophoresis technique could be achieved in 10 minutes using a prototype embodiment of the invention. The saving in time can arise because in current imaging techniques the images of bands identified by conventional labels is heavily smeared by the isotropic emission characteristics of the labels, whereas in embodiments of the present invention the image of a nucleic acid band is substantially the same size as the band, smeared only by diffraction.

Embodiments of the invention can further provide an improved detection sensitivity and signal-to-noise ratio in sub-microlitre sequencing and imaging.

Embodiments of the invention can allow pre-programmed sequence recognition masks to accompany an installation, so that in principle a "yes-no" answer could be obtained by relatively untrained end-users. A sequence triage system results which, coupled to the potentially low cost of this technology, could result in the use of apparatus according to embodiments of the invention in doctors' surgeries, schools

and even on individual researchers' desks.

Embodiments of the invention will now be described, by way of example only, with reference to the accompanying drawings in which:

Figure 1 is a schematic perspective view of part of an analyser according to an embodiment of the invention;

Figure 2 is a schematic plan view of an array of analyser channels according to an embodiment of the invention;

Figure 3 is an enlarged view of two supply wells;

Figure 4 is a schematic cross sectional view of an analyser operating at a UV wavelength of about 260 nm;

Figure 5 is a schematic cross sectional view of an analyser channel operating at a UV wavelength of about 200 nm; and

Figure 6 is a schematic graph illustrating the signal to noise performance of prototype embodiments of the invention.

Referring now to Figure 1, an analyser according to embodiments of the invention comprises an array of channels 10 formed as the substrate is grown or deposited (e.g. by CVD) or etched by excimer laser ablation into a substrate 20 of diamond, sapphire (preferably coated in nanocrystalline diamond) or polymer, possibly deposited on a substrate of a material such as silicon. The channels are at least partially filled by a polyacrylimide gel and are subjected to an electric field along a longitudinal channel direction.

Under the influence of the electric field, DNA samples injected at one end of a channel progress along the channel. Components of the sample progress at a velocity dependent on the molecular weight (often expressed as a number of "base pairs" for DNA samples) of the component.

Ultraviolet light from a light source (not shown in Figure 1) is directed onto the channels, and transmitted light is imaged by a pixel array 30 of ultraviolet light detectors at the other side of the channels, in effect imaging the shadows of DNA components or bands as they pass along the channels.

At a wavelength of 253.9 nm, a mercury lamp can conveniently be used as the light source.

Each channel is less than 250 μm deep (preferably about 150 μm deep), less

than 200 μm wide (preferably 50 μm wide) and 18 mm long. The DNA samples are driven by the electric field from one end of the channel towards the other, and then the polarity of the electric field is reversed so as to drive the samples in the other direction. This process is repeated many times - e.g. several hundred times and the results averaged.

Results can be obtained by detecting the times at which fragments pass a particular point in the channel, or alteratively by performing a Fourier or other transform on the combined output of the array of spaced pixel detectors to detect velocities along the channel directly (see PCT/GB98/00645). The output of the analyser is thus a velocity map or distribution, whereby lower weight fragments have a higher velocity along the channels than higher weight fragments. Alternatively, an image of the separated components can be generated using an array of pixel detectors,. as in PCT/GB96/01121 and analysed directly.

A polymer or diamond lid can be positioned over the channels to avoid contamination.

Figure 2 is a schematic plan view of an array of channels embodied on a substrate about 20 mm x 20 mm in area. A high voltage (HT) source 40 is connected to a tree structure of electrodes 50 at each end of the channels so that an electric field is applied along each channel. Typically the potential difference between ends of the channels may be about 3 kV.

Supply wells 60 are provided to inject DNA samples into each of the channels. An enlarged view of some supply wells is provided in Figure 3, showing that they are formed as substantially circular areas of etched material connected to the channels. They can be filled with a robotic micropipette apparatus available from Evotec GmbH.

So, Figure 2 shows an array of a large number of parallel separation electrophoresis microchannels with associated dendritic branching to and from a buffer well with DNA input and output wells on each channel. DNA may be switched between wells and separation channels using electric fields from suitably positioned electrodes (not shown). In the separation channels it is separated under an electric field as it permeates the electrophoresis gel in the channel - which might be nominally agarose of poly (acrylimide) but other materials are also suitable, such as

alcogel or hydrogel, so that individual lines corresponding to lengths of DNA become distinct.

Flushing the equipment can be carried out rapidly between input plugs. It is also relatively easy to input a solution to multiple plugs.

Figures 4 and 5 schematically illustrate two embodiments of the invention in cross section, one for use at a UV wavelength of about 200 nm (Figure 4) and one for use at about 260 nm, possibly 253 nm using light from a mercury lamp (Figure 5).

In each case, channels 10 are etched into a diamond substrate 20. A UV source 100 operating at the relevant wavelength is positioned so as to direct UV light onto the analyser.

In Figure 4, the lower surface 110 of each channel is made at least partially non-transparent by a treatment such as hydrogenation. When UV light impinges on the surface 110, electron-hole pairs are generated. These can be detected as a photocurrent I by connecting a dc voltage between the surface 110 and a deposited metal layer on a top surface 120 of the substrate. The resulting photocurrent can then be amplified by an amplifier 130.

The signal connections can be made to the surface 110 by depositing an electrically conductive track up the inside of each channel.

The calculation or detection of the concentration of material components between the source and the detector is relatively straightforward, being the difference in detected light levels with and without the band in the way. This is carried out by data processing apparatus such as a general purpose computer (not shown) arranged to receive the output from the detectors.

The source and/or detector can be at a single position with respect to each channel, or can be formed as an array of pixel detectors to image multiple positions on each channel at once.

The advantages of operation at 200 nm are that the absorption of the light by the DNA fragments is about 10 times higher than that at 253 nm. However, this is weighed against the convenience of operation at 253 nm as a simple source (the mercury lamp) can be used.

Figure 5 shows a similar arrangement for operation at 253 nm (or, for

example, at any wavelength near to 260 nm). Here, a confocal focusing arrangement is used, whereby a microlens fashioned during the etching process onto a diamond lid 130 between each channel and the UV source 100 acts to focus the incident light through the contents of the channels and onto a metallised area 140 at the other face of the diamond substrate. Alternatively, of course, if the positions of the source and detector were swapped, the focusing formations could be formed on what is drawn as the underside of the substrate. Again, electron-hole pairs are created and a photocurrent can be detected by applying a potential difference between a metallised layer 120 and the metallised area 140.

The systems of Figures 4 and 5 can be operated at different wavelengths, and in particular the system of Figure 4 could be operated at about 200 nm and that of Figure 5 at about 260 nm.

Diamond as a substrate has a number of particular advantages:

(i) it is chemically inert, hydrophobic and easily cleaned, e.g. using nitric acid;

(ii) it has good thermal conductivity, about 5 times better than copper, so that in embodiments of the invention the entire analyser can be cooled by a Peltier cell at one face of the device;

(iii) it is relatively transparent to UV light, and in particular is one of the most transparent materials known at 257 nm;

(iv) its surface can be made non-transparent when required, e.g. by hydrogenation;

(v) it has a high breakdown voltage - at least 10^7 Vcm^{-1};

(vi) it has a very high refractive index, so that surface or other features formed in the diamond can provide at least partial light concentration.

Figure 6 is a schematic graph showing the signal to noise (S/N) performance obtainable with embodiments of the invention. A signal to noise ratio of 20:1 is obtained for 2 ng / µL DNA.

CLAIMS

1. An analyser comprising:

a substrate of diamond, sapphire or a polymer material;

an array of one or more elongate capillary channels formed in the substrate;

means for driving a sample to be tested along one or more of the channels whereby the velocities of components of the sample along the channels depends on the relative molecular weights of those components;

a radiation source and a radiation detector disposed on either side of the channel array so as to detect the presence of material in the channels as interruptions in the radiation path between the radiation source and the radiation detector.

2. An analyser according to claim 1, in which the substrate is formed of diamond.

3. An analyser according to claim 1, in which the substrate is formed of sapphire having a coating of nanocrystalline diamond.

4. An analyser according to any one of claims 1 to 3, in which the channels are less than 250 μm deep.

5. An analyser according to claim 4, in which the channels are less than 150 um deep.

6. An analyser according to any one of the preceding claims, in which the channels are less than 200 μm wide.

7. An analyser according to any one of the preceding claims, in which the channels are less than 100 μm wide.

8. An analyser according to any one of the preceding claims, in which the radiation source comprises an ultraviolet light source.

9. An analyser according to claim 8, in which the ultraviolet light source is operable to generate ultraviolet light at a wavelength of about 260 nm or about 200 nm.

10. An analyser according to claim 8 or claim 9, in which focusing formations are formed on the substrate to at least partially focus the ultraviolet light onto the interior of each channel.

11. An analyser according to claim 10, in which the focusing formations, the channels and the radiation detector are arranged so that the interior of each channel is substantially mid-way between the focusing formations and the radiation detector.

12. An analyser according to any one of the preceding claims, in which the substrate of diamond, sapphire or a polymer is formed on a further substrate of a semiconductor material, the radiation detector being fabricated on the further substrate of semiconductor material.

13. An analyser according to claim 12, in which the semiconductor material is silicon.

14. An analyser according to claim 11 or claim 12, in which the radiation detector comprises an array of pixel detectors formed on the further substrate.

15. An analyser according to any one of claims 1 to 11, in which the radiation detector comprises an array of obscured regions on the substrate under the channels, and means for detecting an electric current formed by electron-hole pair generation at the obscured regions.

16. An analyser according to claim 15, in which the regions are formed at a lower surface of each channel.

17. An analyser according to claim 15, in which the regions are formed at a lower surface of the substrate substantially beneath each channel.

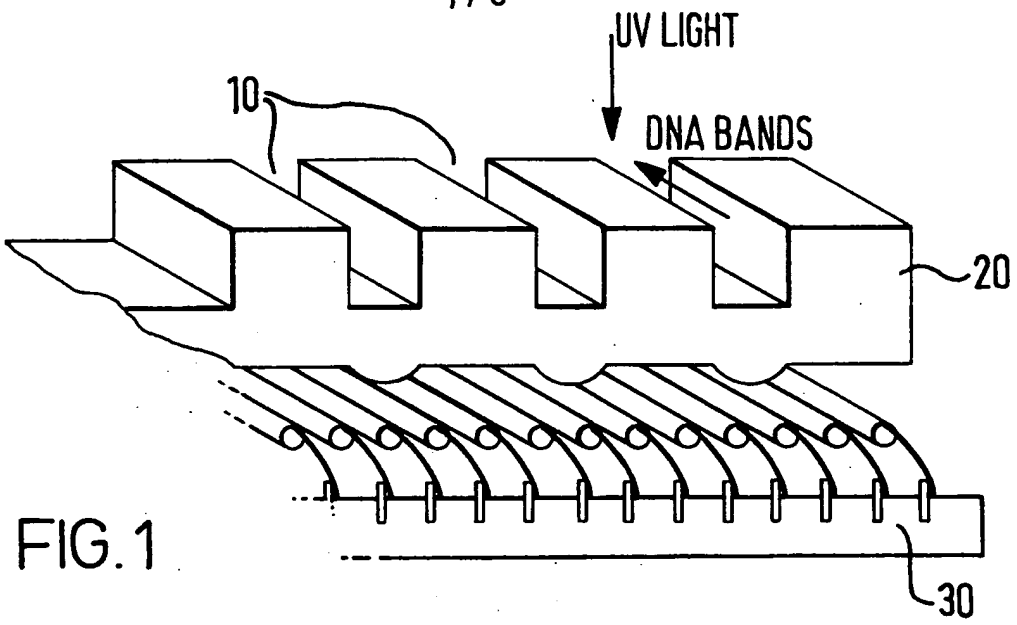


FIG. 1

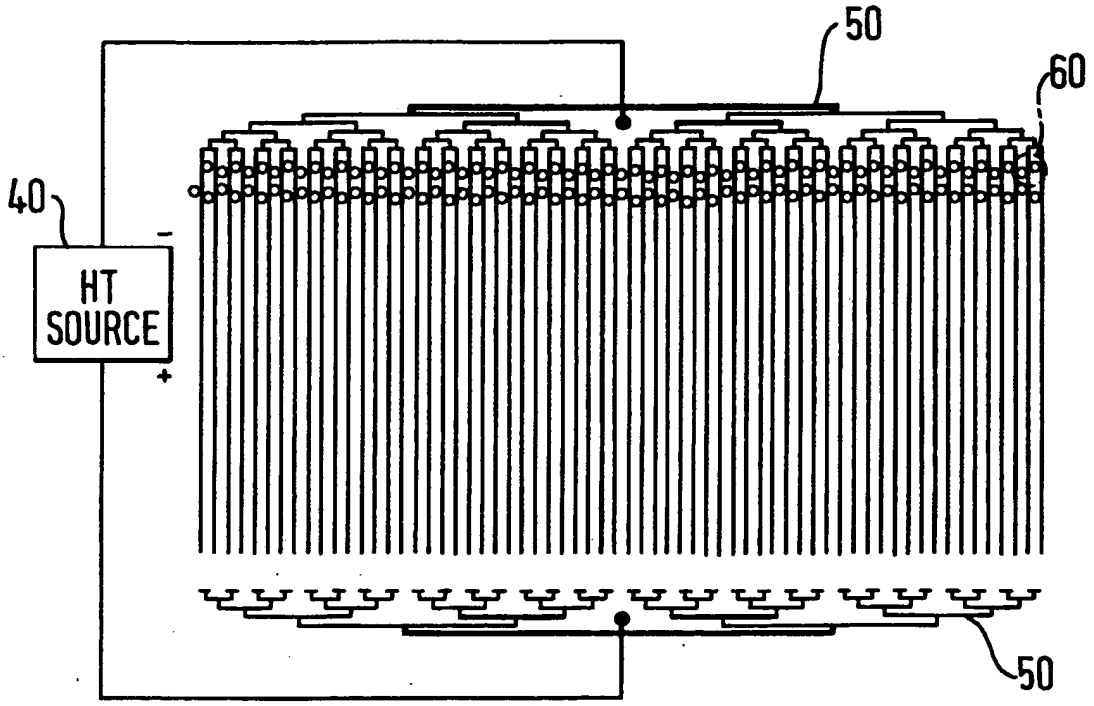


FIG. 2

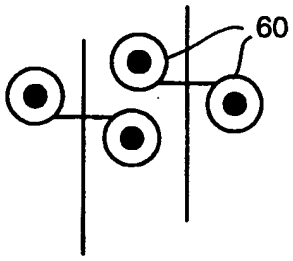

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FIG.3

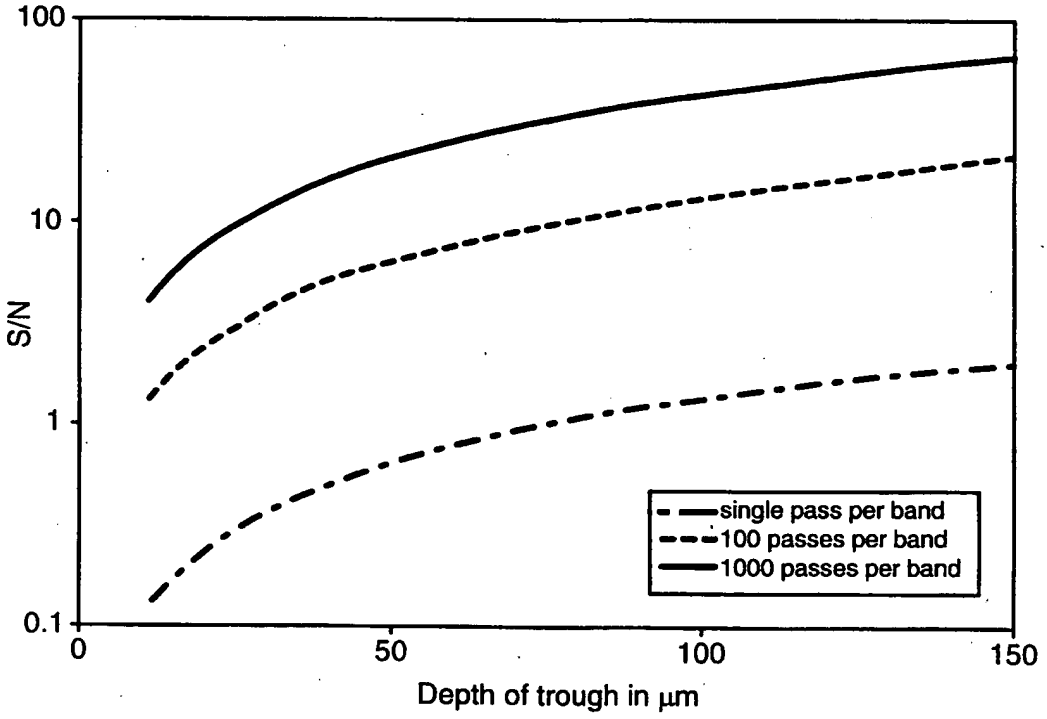

100
10
1
0.1
S/N
0
50
100
150
Depth of trough in μm
single pass per band
100 passes per band
1000 passes per band

FIG.6

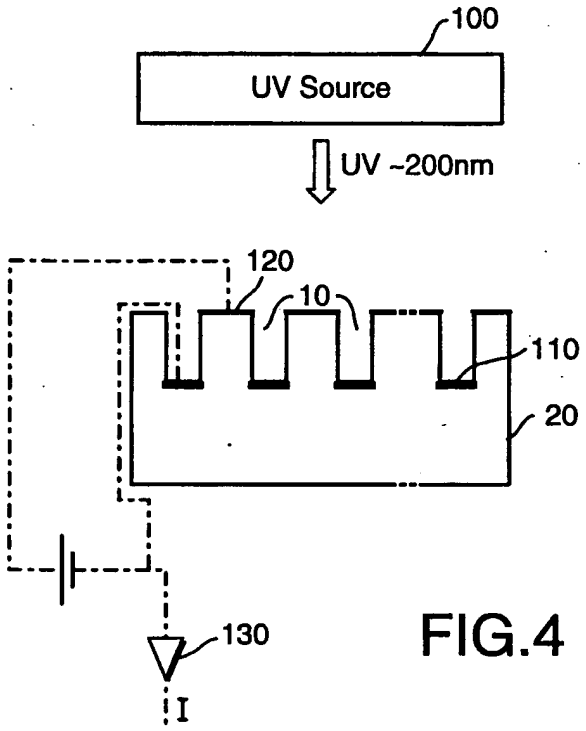

100
UV Source
UV ~200nm
120
10
110
20
130
I

FIG.4

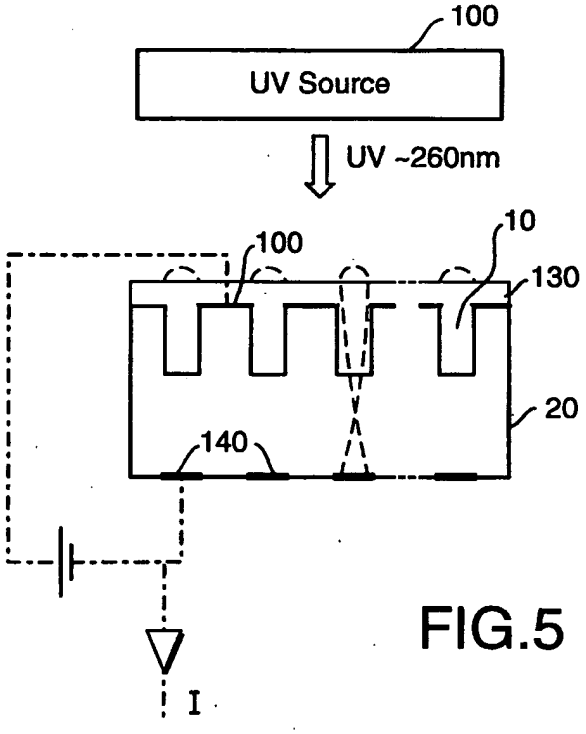

100
UV Source
UV ~260nm
100
10
130
20
140
I

FIG.5

INTERNATIONAL SEARCH REPORT

Inte onal Application No
PCT/GB 99/00742

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N27/447

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 708 331 A (HEWLETT PACKARD CO) 24 April 1996 see page 4, line 21 - page 5, line 14 see page 6, line 1 - page 10, line 26	1,2
X,P	WO 98 10122 A (UNIV NORTHEASTERN) 12 March 1998 see the whole document	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
28 May 1999	30/06/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Müller, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Intl Ional Application No
PCT/GB 99/00742

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	MACDONALD J H ET AL: "A CCD system for UV imaging of biomolecules" NUCLEAR INSTRUMENTS & METHODS IN PHYSICS RESEARCH, SECTION - A: ACCELERATORS, SPECTROMETERS, DETECTORS AND ASSOCIATED EQUIPMENT, vol. 392, no. 1-3, 21 June 1997, page 227-232 XP004090959 see page 227, left-hand column - page 229, right-hand column; figure 2 -----	1,8-11, 14

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